Antibiotics Susceptibility of Salmonella Isolates from Widal Test Positive Patients at the Federal Medical Center, Gusau.

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Abstract
***Background:** Widal test and antibiotics susceptibility testing are routine procedure, used in developing countries, for the diagnosis of endemic typhoid but these need to be monitored because of varying bacterial sensitivity and changing anti-typhoid drug efficacy pattern respectively.*
***Methods:** Consequently blood samples were collected from a total of 83 febrile patients who showed clinical signs of typhoid in Gusau. The samples were examined for S. typhi infection by the Widal tube agglutination and Blood culture methods. Antibiotics susceptibility test was also carried out on the S. typhi isolates.*
***Results**: The result shows that there was a significant difference ($P < 0.05$) between samples (70 or 84.3%) that were widal test positive only and those that were both widal and culture positive (29 or 25.9%). S. typhi 'O' and 'H' antibody titre of 1:80 and 1:160 as well as 1:40 and 1:160 were shown in 35 (42.2%) and 15 (18.1%) of samples respectively, while 13 (15.7%) had 1:20 antibody titer for both antigens. The reciprocal titer of 1: 80 and 1:320 for 'O' and 'H' antigens was recorded for 15 (18.3%) of the samples. Isolates showed less susceptibility 54 (18.6%) but more resistance (236 or 81.4%) to all anti-bacteria tested. Ampiclox, Ciprofloxin, Chloramphenicol and Erythromycin were the most sensitive with susceptibility proportion of 14 (46.7%), 10 (34.5%) and 7 (23.3%) respectively.*
***Conclusion:** This investigation shows a high distribution rate of endemic typhoid antigens, among patients in study area and the resistance of S. typhi isolates to the more available, standard and cost effective drugs such as chloramphenicol, probably due to public abuse of this drug.*

Introduction

Typhoid fever is a bacterial illness caused by *Salmonella typhi*, a gram-negative rod commonly excreted in the feces and urine of man, and also found in the blood and gallbladder[1]. A Single Widal test on serum during acute illness together with the presence of characteristic clinical features of typhoid has been the adopted option of diagnosis in most clinics and hospitals in developing countries where typhoid and paratyphoid are endemic.[2] The Gusau Federal Medical Center (FMC), which is the site of this study, is one of such hospitals in Nigeria where a single widal test is used in routine diagnosis of typhoid fever. This study is a pioneer attempt to have on record the susceptibility pattern of *S. typhi* isolates to some antibiotics commonly obtainable at the market and in most clinics and hospitals in Gusau. This is informed by the need for a reference point in the diagnosis and proper antibiotics treatment of typhoid in study area[3, 4]. The study is also essential because of many cases of multiple drug resistance by *S. typhi* that has been documented in many parts of the world including Nigeria[3,4,5] and attributed to widespread and indiscriminate use of poor choice of anti-typhoid drugs, especially in areas such as Gusau where the effective drugs had not been sorted bacteriologically. It is therefore the aim of this work to isolate *S. typhi* from blood of patients suspected of typhoid fever and to test the isolates with the commonly available antibiotics sold at the Markets or dispensed at most clinics in Gusau. The incidence of typhoid fever will be greatly reduce if routine isolation of *S. typhi* for susceptibility testing is encouraged to guide the right choice of drug and adequate treatment of typhoid[4, 5, 6].

Materials and Methods

Blood was collected from eighty-three (83) febrile patients who reported at the Federal Medical Center, Gusau, within the first ten days of fever. The eligible subjects had also not started taking treatment but had been clinically diagnosed as having typhoid fever. Each patient was tested when 7-5ml of blood was collected by venipunture, a standard procedure[4, 5, 6]. 2mls of the blood was allowed to clot at room temperature and centrifuged for 5 minutes at 3000rpm (rotation per minute) to obtain the serum. Widal test was conducted on the serum following the manufacturer's instruction (Antec Diagnostic Products, 2005)[4,6]. 5-3ml of the blood, used for sample culture, was dispensed into McCartney bottles containing 10mls thioglycholate broth. These were inoculated into Salmonella-Shigella (SS) medium, and then incubated at 37°C for 24 hours. Samples without growth were re-incubated at the same temperature for 7 days and sub-cultured into SS medium before being discarded. Pale non-lactose fermenting colonies were picked from the SS plates as positive and confirmatory biochemical tests including Hydrogen Sulphide Production, Lysine decarboxylation, glucose, lactose, motility, indole, and oxidase tests were performed accordingly[4,5]. Isolates confirmed by biochemical test were further tested for their susceptibility to ten antimicrobial agents using MIC (Minimum Inhibitory Concentration) test[4] at the following discs contents: Ampiclox 30Fg, Ciprofloxin 10Fg, Norfloxaxin 30Fg, Gentamycin 10Fg, Lincocin 30 Fg, Streptomycin 30Fg, Rifampin 10Fg, Floxapen 30Fg, Erythromycin 30Fg, and Chloramphenicol 20Fg. Nutrients Agar plates were used for the sensitivity test and these were incubated at 37°C for about 24 hours. The minimum inhibition concentration (MIC) was the least antimicrobial concentration that yielded no visible growth[4,5,6].

Results

Sera from 35 (42.2%) of the 83 blood samples examined had reciprocal titer level of 1:80 and 1:160 for 'O' and 'H' antigens respectively, and out of these 15 (45.8%) were culture positive. Similarly sera from 20 (24.1%) of the blood samples which had a widal titer of 1:40 and 1:160 for 'O' and 'H' antigen respectively, contained 8 (53.3%) samples which yielded cultural growth of *Salmonella typhi*. In the follow-up result, 6 (46.2%) of the 15 (18.1%) samples which had 'O' and 'H' antigens levels of 1:80 and 1:320 respectively were culture positive. Although all of the Widal test negative samples were also negative for culture, there was significant difference ($P < 0.05, df = 3: 8.08$) between the widal and culture test positive samples respectively (Table 1).

As indicated in the result of the susceptibility pattern of *S. typhi* isolates to standard concentrations of the antibiotics tested (Table 2), none of the isolates was completely susceptible to any of the anti-microbial agents used (Table 2). Generally, resistance was 81.4% (x = 236, n= 290) compared to susceptibility of 54 or 18.6% of the isolates (Table 2). However, the most active anti-bacteria included Ampiclox and Ciprofloxin to which 14 (46.7%) of the isolates were respectively susceptible. Similarly 10 (34.5%) and 7 (23.3%) of the isolates were susceptible to the next most effect drugs which were Chloramphenicol and Erythromycin respectively. Among the agents that showed the least activity against the isolates were Gentamycin, Lincocin, Rifampin and Floxapen, to which resistance was 96.6% respectively, while 27 (93.1%) and 26 (89.7%) of the isolates were resistant to Streptomycin and Norfloxacin respectively.

Table 1. Results of the Widal test and Blood culture

S. typhi antigens: 'O' Titre	'H' Titre	No. (%) of the Widal Positive Patients	No. (%) samples of *S. typhi* Positive blood culture.
1:20	1 3	(1 5 . 7)	
1:20	0(0.0)		
1:40		20 (24.1)*	
1:160	8(53.3)		
1:80		35 (42.2)*	
1:160	15(42.8)		
1:80		15 (18.1)*	
1:320	6(46.2)		
	70* (84.3%, n=83)	29 (25.9%)	

*Widal positive patients

$X^2 = 8.08$, $P < 0.05$

Table 2. Concentrations and sensitivity rates of *S. typhi* isolates to the antibiotics.

Antibiotics	Conc. (µg)	No. of Isolates sensitive (%)	No. of Isolates resistant (%)
Ampiclox	30	14 (4 6.7)	15 (51.7)
Ciprofloxin	10	14 (46.7)	15 (51.7)
Norfloxacin	30	3 (10.3)	26 (89.7)
Gentamycin	10	1 (3.4)	28 (96.6)
Lincocin	30	1(3.4)	28 (96.6)
Stertomycin	30	2 (6.9)	27 (93.1)
Rifampin	10		

Ampiclox	**30**	**14 (4 6.7)**	**15 (51.7)**
Ciprofloxin	**10**	**14 (46.7)**	**15 (51.7)**
Norfloxacin	**30**	**3 (10.3)**	**26 (89.7)**
Gentamycin	**10**	**1 (3.4)**	**28 (96.6)**
Lincocin	**30**	**1(3.4)**	**28 (96.6)**
Stertomycin	**30**	**2 (6.9)**	**27 (93.1)**
Rifampin	**10**	**1 (3.4)**	**28 (96.6)**
Floxapen	**30**	**1 (3.4)**	**28 (96.6)**
Erythromycin	**30**	**7 (23.3)**	**22 (75.9)**
Chloramphenicol	**20**	**10 (34.5)**	**19 (65.5)**
Total		**54 (18.6%)**	**236 (81.4%)**

Discussion

Since all the febrile patients examined for typhoid in this investigation tested positive for anti-Salmonella antibody, it may be suggested that this organism is important in the aetiology of pyrexia in study area. Previous study has shown that typhoid is widespread in the neighboring community of Sokoto[3]. However, the subjects were not investigated for non-typhoidal agents of fever and so it could not be confirmed that typhoid was the sole aetiological agent of fever in this study. It is noteworthy that intestinal and malaria parasites are also common agents of fever in developing communities[7,8]. High rates and levels of anti-typhoid antibodies as seen in this study is characteristic of endemic typhoid in exposed communities of Nigeria[9,10]. Although a single widal test offered important clue to typhoid infection in this study, the diagnostic value of this test is clearly presumptive, because of the cases of positive widal that tested negative for the blood culture. Other studies have shown that the isolation of *S. typhi* in blood, stool or urine culture is more reliable to give the definitive diagnosis of typhoid[11, 12]. However, Widal test is useful and remains the only available test for typhoid, especially in some health care facilities and district laboratories of African countries, as in study area, where cultural test is problematic or unaffordable. Sherwai et al[13] working in India had suggested the Typhoid test, an immunodot ELIZA as an alternative, with better sensitivity and specificity for typhoid than widal. Compared to culture and other serological tests, however, Widal test is more frequently performed in endemic areas of Africa because it is less expensive, easier to perform, require no special skill or equipment and the result is ready in minutes instead of days as with culture. A cautious use of widal test with more careful interpretation and possibly, in combination with a follow-up cultural confirmatory test is recommended in study area because of the significant difference between widal and culture positive results in this report. Health workers should also be guided by the fact that the chances of a widal positive case to be culture positive is about 3:1 (83:29) in study area.

Typhoid, in this study has shown resistance to all tested drugs including Chloramphenicol which is the drug of choice for treatment of typhoid in many developing countries because of its efficacy, availability and affordability[14, 15]. However, it is contraindicated in some patients in whom it induces aplastic anaemia and also limited by typhoid resistance to it and other first line drugs including ampicillin and amoxacillin[14, 15]. *S. typhi* had shown acquired multi-drug resistance to many other anti-bacteria including the flouroquinolines which are widely regarded as optimal drugs for treatment of typhoid fever in adults[15, 16, 17]. Mandatory antibacterial sensitivity testing is therefore recommended in study area because of varying degrees of sensitivity shown in this study and also because of reported cases of re-emergent sensitivity to standard anti-typhoid which had previously lost efficacy[16]. It had been recommended in some endemic communities in Nigeria, that in all cases of positive cultures, antibiotic sensitivity to chloramphenicol, ampicillin, cotrimoxazole and norfloxacin (or ciprofloxin) should be considered[10,17]. Antibiotic sensitivity testing is also useful because treatment of typhoid with appropriate drugs remains a viable control option in the absence of effective anti-typhoid vaccine and also because endemic typhoid is characteristic of many communities in the developing world[14, 18, 19]. This level of occurrence of typhoid which is a food and water-borne disease is an indication of prevalent poor food hygiene habit and/or insanitary drinking water supply in study area[14, 18]. Typhoid resistance to antimicrobials as seen in this and other studies is a global health problem[3, 12, 20] which has been largely attributed to the ease of procurement and indiscriminate use of these drugs by patients, without due medical consultation. It is therefore of public health importance that this trend is controlled, possibly by aggressive health education and enforcement of compulsory typhoid sensitivity testing for appropriate choice of drugs and effective treatment of this preventable bacterial infection.

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